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PHARMACOLOGY

Evaluation of Oritavancin Dosing Strategies against Vancomycin-Resistant *Enterococcus faecium* Isolates with or without Reduced Susceptibility to Daptomycin in an *In Vitro* Pharmacokinetic/Pharmacodynamic Model

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ABSTRACT The clinical development of nonsusceptibility to the lipopeptide antibiotic daptomycin remains a serious concern during therapy for infections caused by vancomycin-resistant *Enterococcus faecium* (VREfm). The long-acting lipoglycopeptide oritavancin exhibits potent *in vitro* activity against VREfm, although its safety and efficacy for treating clinical VREfm infections have not been established. In this study, novel dosing regimens of daptomycin and oritavancin were assessed against both VREfm and daptomycin-nonsusceptible VREfm isolates in an *in vitro* pharmacokinetic/pharmacodynamic model.

KEYWORDS *E. faecium*, PK/PD, VRE, VanA, daptomycin, oritavancin, vancomycin-resistant

Enterococcus faecium is among the leading causes of nosocomial infections and is listed as an ESKAPE (*E. faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species) pathogen, a group of six bacterial species against which there is an urgent need for new therapies due to the prevalence of multidrug resistance (1). For infections caused by vancomycin-resistant *Enterococcus faecium* (VREfm), high-dose regimens (≥8 mg/kg of body weight/day) of the lipopeptide daptomycin are often used despite a lack of efficacy and safety data from adequate and well-controlled clinical trials and a lack of regulatory approval for this organism. High-dose daptomycin regimens may help improve efficacy and limit resistance development by VREfm (2, 3). However, case reports have described the development of reduced susceptibility to daptomycin in patients receiving doses of 10 mg/kg/day (2, 4). We have recently shown that reduced susceptibility to daptomycin occurred in VanA-positive VREfm isolates exposed to daptomycin at free drug levels expected from 12-mg/kg/day dosing in an *in vitro* pharmacokinetic/pharmacodynamic (PKPD) model (5). In contrast, exposure to the lipoglycopeptide oritavancin at free drug exposures expected from a single 1,200-mg dose eradicated the tested VREfm isolates (5). In this study, we explore the pharmacodynamic activity of a regimen of daptomycin followed by a switch to oritavancin in preventing the emergence of daptomycin-nonsusceptible VREfm in the *in vitro* PK/PD model. Furthermore, oritavancin activity against daptomycin-nonsusceptible VREfm isolates was also assessed.

The VanA-positive VREfm clinical isolates used in this study included B7181440, B7231527, and their respective daptomycin-nonsusceptible mutants, 1440-141-1 and 1527-140-4, which were derived from daptomycin exposure in a previous study (5). Oritavancin (The Medicines Company, Parsippany, NJ) and daptomycin (APIChem Technology Company, Hangzhou, China) reference broth microdilution MICs for each isolate were determined according to CLSI M07-A10 guidelines (6) using the *E. faecalis* quality control isolate ATCC 29212 to confirm appropriate drug and assay performance

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TABLE 1 Pharmacokinetic parameters obtained for the indicated dosing regimens in the *in vitro* PK/PD model

Parameter[a]	Daptomycin, 12 mg/kg/day		Oritavancin, 1,200-mg single dose	
	Targeted[b]	Obtained ± SD	Targeted[c]	Obtained ± SD
fC_{max} (μg/ml)	15.6	15.7 ± 0.8	20.7	20.1 ± 1.7
$fAUC_{0-24}$ (μg · h/ml)	171	164 ± 19.6	178	156 ± 17.2
$t_{1/2}$ (h)	8	8.4 ± 1.5	ND[d]	ND

[a] fC_{max}, free peak concentration; $fAUC_{0-24}$, area under the concentration-time curve from 0 to 24 h; $t_{1/2}$, half-life.
[b] The targeted PK values for daptomycin were derived from a study by Benvenuto et al. (15) and the prescribing information (8), assuming 91.5% protein binding. The daptomycin $t_{1/2}$ in the *in vitro* PK/PD model was determined by nonlinear regression analysis using the GraphPad Prism 6 software. The targeted $fAUC_{0-24}$ was calculated using a simulated daptomycin concentration-time profile (Prism 6) that respects the targeted PK parameters (fC_{max}, 15.6 μg/ml; $t_{1/2}$, 8 h).
[c] The targeted PK values for oritavancin were derived from a study by Rubino et al. (16), assuming 85% protein binding. The targeted $fAUC_{0-24}$ values were calculated (Prism 6) from the mean oritavancin concentration-time profile obtained from population PK modeling (16).
[d] ND, not determined.

(7). Exponentially growing bacteria were used at a starting inoculum of 10^6 CFU/ml in the *in vitro* PK/PD model. *In vitro* PK/PD modeling was performed for 72 h, as previously described (5) in a dilutional one-compartment model to simulate free drug exposures expected from 12 mg/kg/day daptomycin (assuming protein binding of 91.5%, a free peak concentration [fC_{max}] of 15.6 μg/ml, a half-life [$t_{1/2}$] of 8 h, and a free area under the concentration-time curve from 0 to 24 h [$fAUC_{0-24}$] of 171 μg · h/ml) (8, 9) or a single 1,200-mg dose of oritavancin (assuming protein binding of 85% an fC_{max} of 20.7 μg/ml; alpha, beta, and gamma $t_{1/2}$ of 2.3 h, 13.4 h, and 245 h, respectively; and a $fAUC_{0-24}$ of 178 μg · h/ml) (10); regimens that included either a switch to oritavancin or the addition of a second dose were administered by a 3-h infusion at 24 h. After 5 and 24 h of drug exposure, cultures in the central flask were transferred to new sterilized systems, as previously described (5) to ensure that only drug-exposed bacteria remained and, when applicable, to facilitate a change in medium used for daptomycin exposure (cation-adjusted Mueller-Hinton broth supplemented with 50 μg/ml Ca^{2+}) to medium for oritavancin exposure (cation-adjusted Mueller-Hinton broth supplemented with 0.01% polysorbate-80) for exposures that included a switch to oritavancin at 24 h. Bacterial viability was determined at the indicated times by serial dilution plating, as described previously (11). MICs of the derived mutants that survived drug challenge were determined by reference broth microdilution (6) before and after serial passage on nonselective medium (cation-adjusted Mueller-Hinton agar) for 5 days to assess the stability of the susceptibility changes. Daptomycin and oritavancin concentrations in the *in vitro* PK/PD model were determined using previously described bioassay and fluorescence polarization methods, respectively (5). Statistical comparisons in mean changes in bacterial viability (log CFU per milliliter) relative to the inoculum were compared by a *t* test ($P < 0.05$). Bacterial genomic DNA (GenElute bacterial genomic DNA kit; Sigma-Aldrich, Oakville, Ontario, Canada) was sequenced using the PacBio system at the McGill University and Génome Québec Innovation Centre (Montreal, Québec, Canada). Genomic analysis was done at the Canadian Center for Computational Genomics at the McGill University and Genome Quebec Innovation Centre.

Daptomycin at free drug concentrations expected from 12-mg/kg/day doses (Table 1) was rapidly bactericidal (≥3-log kill relative to the starting inoculum) within 5 h and exhibited sustained killing over 24 h against the two VREfm isolates (Fig. 1). Similar to previous observations (5), regrowth of daptomycin-nonsusceptible mutants occurred with both VREfm isolates: 3 of 4 replicates of B7181440 and 2 of 4 replicates of B7231527 exhibited regrowth at 48 to 72 h. The observed increases in daptomycin MIC were stable and ranged from 4- to 8-fold above the MIC of the respective parental isolates (Table 2). In contrast, no regrowth of either VREfm isolate occurred when a 3-h infusion of oritavancin that targeted free drug concentrations expected from a single

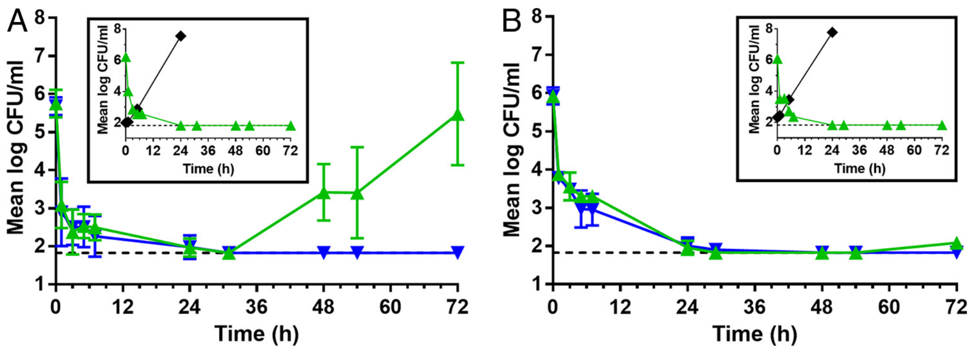

FIG 1 Pharmacodynamic activities of daptomycin at free drug concentrations associated with 12 mg/kg/day for 72 h (green triangles) or daptomycin at 12 mg/kg followed by a switch at 24 h to a single 3-h infusion of 1,200 mg oritavancin (blue inverted triangles) against the clinical isolates of VanA-positive VREfm B7181440 (A) and B7231527 (B) in an *in vitro* PK/PD model over 72 h. Mean log CFU/ml ± standard deviation values are from two independent experiments done in duplicate. The inset in panel A depicts the single occurrence of eradication of B7181440 following exposure to daptomycin. The inset in panel B depicts the eradication of B7231527 in two replicates following exposure to daptomycin. Both insets also show control cultures (black diamonds) for each isolate using the flow rates for oritavancin to supply fresh drug-free medium over 24 h. The dashed line indicates the limit of detection (<66.7 CFU/ml).

1,200-mg dose (Table 1) was administered 24 h after an initial dose of daptomycin. Although exposure to the daily daptomycin regimen resulted in mutants with cross-reduced susceptibility to oritavancin (4-fold increase in oritavancin MIC), our results demonstrate that the administration of oritavancin 24 h after an initial dose of daptomycin

TABLE 2 Daptomycin and oritavancin broth microdilution MICs against VREfm parental isolates and daptomycin-nonsusceptible derived mutants that survived daptomycin or oritavancin challenge in the *in vitro* PK/PD model

Parental VREfm isolate or derived mutant[a]	MIC[b] (μg/ml)	
	Daptomycin	Oritavancin
B7181440	2	0.06
1440-145-3	16	0.25
1440-145-4	16	0.25
1440-150-4	16	0.25
7231527	4	0.5
1527-153-3	16	1
1527-153-4	16	1
1440-141-1	16	0.5
1440-151-1	16	1
1440-151-2	16	1
1440-151-3	16	1
1440-151-4	16	1
1440-156-3	32	0.5
1440-156-4	32	0.5
1440-157-3	16	0.5
1440-157-4	32	0.5
1527-140-4	16	1
1527-158-3	32	0.5
1527-158-4	32	0.5
1527-159-3	16	1
1527-159-4	16	1
1527-160-1	16	1
1527-160-2	32	1
1527-160-3	16	1
1527-160-4	16	1

[a]All but B7181440 and B7231527 are daptomycin-nonsusceptible-derived mutants. Three of four replicates of B7181440 and two of four replicates of B7231527 showed regrowth of derived mutants with reduced susceptibility to daptomycin following daptomycin exposure in the *in vitro* PK/PD model. MICs of the derived mutants were unchanged (within 2-fold) following 5 days of passage on nonselective media.
[b]Modal MICs are presented from ≥2 independent determinations.

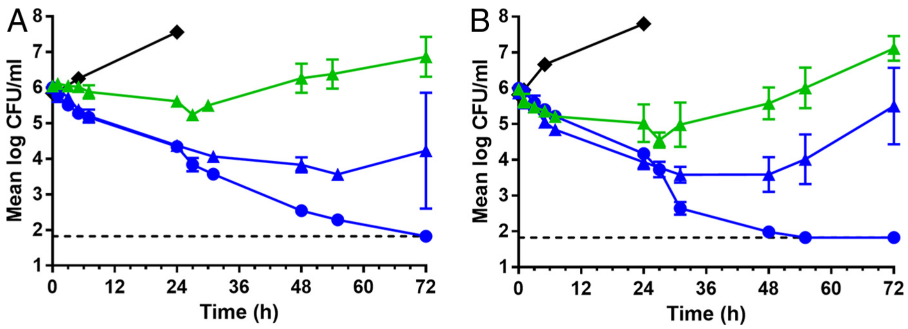

FIG 2 Pharmacodynamic activities of daptomycin at free drug exposures associated with 12 mg/kg/day daptomycin (green triangles), a 3-h infusion of a single 1,200-mg dose of oritavancin (blue triangles), or two 3-h infusions of 1,200 mg of oritavancin (blue circles; first infusion started at time zero, second infusion started at 24 h) against daptomycin-nonsusceptible VREfm 1440-141-1 (A) and 1527-140-4 (B) in an *in vitro* PK/PD model over 72 h. Mean log CFU/ml ± standard deviation values are from two independent experiments done in duplicate. Control cultures are shown for each isolate (black diamonds) using the flow rates for oritavancin to supply fresh drug-free medium over 24 h. The dashed line indicates the limit of detection (<66.7 CFU/ml).

eliminated the few (≤66.7 CFU/ml) mutants that survived the initial daptomycin challenge. Thus, switching to oritavancin following initial exposure to daptomycin may represent a novel dosing strategy to eliminate the emergence of reduced susceptibility to daptomycin. However, the safety and efficacy of this potential resistance avoidance strategy remain to be established in controlled clinical trials.

As limited therapeutic options are available for daptomycin-nonsusceptible VREfm isolates, we determined if oritavancin would be effective against these challenging drug-resistant pathogens. Two daptomycin-nonsusceptible VREfm mutants (1440-141-1 and 1527-140-4) that were previously derived from exposures of their cognate parental isolates (B7181440 and B7231527) to daptomycin at free drug concentrations expected from 12 mg/kg/day in the *in vitro* PK/PD model (5) were selected for further study. Mutant 1527-140-4 (daptomycin MIC, 16 μg/ml) contains a single nucleotide polymorphism that encodes an amino acid change (Val129Glu) in LiaS, which is part of the LiaFSR three-component system that has been implicated in reduced susceptibility to daptomycin (12). Mutant 1440-141-1 (daptomycin MIC, 16 μg/ml) also exhibits cross-reduced susceptibility to oritavancin (oritavancin MIC, 0.5 μg/ml) that is conferred by an unknown mutation (not in the *liaFSR* cistron). To identify genetic mutations that may be involved in the cross-reduced susceptibility, whole-genome sequencing of 1440-141-1 and its parent B7181440 was performed. A single nucleotide polymorphism in the *bceB* gene was identified that results in a nonsynonymous mutation (Ser171Pro) in BceB, a bacitracin transporter which is known to be activated in response to cell envelope stress induced by exposure to antimicrobial peptide antibiotics (13, 14). Therefore, it is of future interest to elucidate the mechanism by which this gene confers reduced susceptibility to both daptomycin and oritavancin.

In the *in vitro* PK/PD model, exposure of the VREfm isolates 1440-141-1 and 1527-140-4 to daptomycin at free drug concentrations associated with 12 mg/kg/day reduced cell viability by 0.5 ± 0.1 and 1.0 ± 0.7 log CFU/ml at 24 h, respectively, relative to the starting inocula; subsequent regrowth of both isolates was observed over the following 48 h (Fig. 2). That daptomycin exposure (mean fC_{max}, 15.7 μg/ml) caused initial reductions in bacterial counts of the daptomycin-nonsusceptible mutants may indicate that their discrete daptomycin MICs range between 8 and 16 μg/ml (although the MICs are reported as 16 μg/ml due to the 2-fold dilutions used in broth microdilution assays). Exposure of the daptomycin-nonsusceptible VREfm isolates to a single infusion of oritavancin over 3 h resulted in a significant reductions ($P < 0.05$) in bacterial viability ranging from 1.6 ± 0.2 to 2.0 ± 0.3 log CFU/ml over 24 h but with subsequent regrowth occurring at 72 h (Fig. 2). These findings contrast with the observed eradication of their daptomycin-susceptible parental counterparts (B7181440 and B7231527) following exposure to a single 1,200-mg dose of oritavancin (5). Thus, more than one oritavancin dose may be needed to eradicate daptomycin-nonsusceptible VREfm isolates exhibiting oritavancin MICs of $\geq$0.25 μg/ml. Indeed, the administration of a second 3-h infusion of oritavancin at 24 h resulted in significant ($P < 0.05$) and progressive eradication of both daptomycin-nonsusceptible VREfm isolates to below the limit of detection (<66.7 CFU/ml) at 72 h (Fig. 2). That a second dose of oritavancin reduced the bacterial counts of the isolate exhibiting cross-reduced susceptibility to below the limit of detection suggests that a multiple-dose strategy with oritavancin may be effective against daptomycin-nonsusceptible VREfm. The safety and efficacy of multiple-dose oritavancin remain to be established in controlled clinical trials.

In conclusion, oritavancin may prove to be an important agent in the armamentarium against infections caused by either daptomycin susceptible or -nonsusceptible VREfm isolates. Further studies to identify appropriate dosing strategies for clinical infections are warranted.

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